Gastric inhibitory polypeptide immunoneutralization attenuates development of obesity in mice

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Boylan MO, Glazebrook PA, Tatalovic M, Wolfe MM. Gastric inhibitory polypeptide immunoneutralization attenuates development of obesity in mice. Am J Physiol Endocrinol Metab 309: E1008–E1018, 2015. First published October 20, 2015; doi:10.1152/ajpendo.00345.2015.—Previous reports have suggested that the abrogation of gastric inhibitory polypeptide (GIP) signaling could be exploited to prevent and treat obesity and obesity-related disorders in humans. This study was designed to determine whether immunoneutralization of GIP, using a newly developed specific monoclonal antibody (mAb), would prevent the development of obesity. Specific mAb directed against the carboxy terminus of mouse GIP was identified, and its effects on the insulin response to oral and to intraperitoneal (ip) glucose and on weight gain were evaluated. Administration of mAb (30 mg/kg body wt, BW) to mice attenuated the insulin response to oral glucose by 70% and completely eliminated the response to ip glucose coadministered with human GIP. Nine-week-old C57BL/6 mice injected with GIP mAbs (60 mg/kg BW−1·wk−1) for 17 wk gained 46.5% less weight than control mice fed an identical high-fat diet (P < 0.001). No significant differences in the quantity of food consumed were detected between the two treatment groups. Furthermore, magnetic resonance imaging demonstrated that subcutaneous, omental, and hepatic fat were 1.97-, 3.46-, and 2.15-fold, respectively, lower in mAb-treated animals than in controls. Moreover, serum insulin, leptin, total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides were significantly reduced, whereas the high-density lipoprotein (HDL)/TC ratio was 1.25-fold higher in treated animals than in controls. These studies support the hypothesis that a reduction in GIP signaling using a GIP-neutralizing mAb might provide a useful method for the treatment and prevention of obesity and related disorders.

A 2014 report from the World Health Organization (13) indicated that ~13% of the world’s adult population (11% of men and 15% of women) were obese, defined as a body mass index (BMI) of ≥30 kg/m², and that 39% of adults aged ≥18 years (38% of men and 40% of women) were overweight (BMI 25.0–29.9 kg/m²). Although previously considered a problem associated with high-income nations, the incidence of obesity is now increasing in low- and middle-income countries, particularly in urban settings. Obesity is linked to more deaths worldwide than conditions characterized by being underweight. A report published by the Centers for Disease Control and Prevention in June 2015 (12) stated that 34.9% of adult Americans (or 78.6 million) were obese and that the estimated annual medical cost of obesity in the United States was $147 billion in 2008 US dollars; the medical costs for people who are obese were $1,429 higher than those of normal weight. The prevalence of obesity in the United States increased dramatically during the last 25 years of the 20th Century. In 1991, whereas no individual state in the United States reported obesity prevalence rates >20% (37), only 21 years later in 2012, no individual state reported an obesity prevalence <20% (29). Obesity thus constitutes a major financial liability and was recently ranked as the third highest economic burden created by humans worldwide.

An important factor that must be considered when the cause of obesity is being evaluated is the capacity of humans and all organisms to store nutrients, principally in the form of glycogen and lipids, and to utilize these stores (46). The most critical factor involved in nutrient deposition is insulin, which appears to have evolved from the need to maximize energy efficiency, obviating the requirement to continuously forage for food (33). The role of the gastrointestinal (GI) tract, and GI regulatory peptides in particular, constitutes another important consideration for defining and investigating obesity, since the GI tract represents the route by which all nutrient sources of energy are ingested, processed, and absorbed.

Recent studies by Musson et al. (26) have provided evidence to support the notion that, during the course of evolution, insulin biosynthesis translocated from the intestine to pancreatic islets, which necessitated a messenger from the intestine to complete the so-called “enteroinsular axis”. The eventual development of gastric inhibitory polypeptide (GIP) and other incretins fulfilled this requirement. Early studies demonstrated that, when administered in physiological doses and in the presence of glucose, GIP is a potent stimulator of insulin release by pancreatic islet β-cells (30, 31). Studies in patients with type 2 diabetes mellitus have demonstrated that the insulinotropic properties of GIP appear to be greatly diminished. Although the mechanisms accounting for this observation have not been clearly elucidated, a decrease in pancreatic islet-β cell GIP receptor number, decreased circulating GIP half-life, defective meal-stimulated GIP release, and chronic desensitization of the GIP receptor due to elevated serum GIP levels have all been implicated (39). In its vital role as a physiological incretin, GIP accounts for 65–70% of the postprandial insulin response (40). The additional survival benefit offered by GIP appears to be its ability to not only stimulate insulin release but to possess insulin-mimetic properties, including effects on nutrient homeostasis (36, 37). This physiological redundancy served to ensure the survival of organisms during times when food was in short supply.

GIP is a 42-amino acid polypeptide synthesized in enteroendocrine K-cells, which are located in the intestinal mucosa of the duodenum and proximal jejunum (4, 22, 37). GIP secretion is stimulated by nutrients, including glucose, fat, and hydrogenated protein (5). GIP binds to its G protein-coupled 7-transmembrane domain receptor (GIPR) on target cells (43), and...
GIP signaling stimulates glucose absorption in enterocytes (34), potentiates glucose-dependent insulin release from islet β-cells (44), increases glucose uptake while inhibiting lipolysis in adipocytes (10, 36), increases nutrient uptake into bone, and inhibits bone resorption (28, 47).

The cell-specific activities of GIP are consistent with its role as an efficiency hormone increasing nutrient uptake and storage. Disruption of GIP signaling by genetic deletion of the GIP or GIPR genes (22, 27) by targeted ablation of GIP-producing K-cells (1), by the administration of GIP derivatives that act as competitive antagonists (8), or by vaccination (7) results in less efficient nutrient storage in mice fed a high-fat diet (HFD). Attenuated nutrient storage appears to protect the mice from developing obesity and other abnormalities associated with the metabolic syndrome, including insulin resistance, type 2 diabetes mellitus, fatty liver, and dyslipidemia (7, 22).

These mouse models suggest that interference with GIP-regulated signaling could be exploited to prevent and treat obesity in humans. To this end, a safe and effective method for decreasing GIP signaling must be identified and developed. Since 1986, monoclonal antibody (mAb) therapy has increasingly been recognized as safe and effective for the treatment of human disease (2). In the present studies, we have identified and developed a high-affinity, specific mAb to GIP, which significantly reduced the insulin response to oral glucose in mice without any effect on glycemia. After 17 wk on an HFD, despite no significant differences in food consumption, mice receiving GIP mAbs gained 46.5% less than control mice. Imaging studies demonstrated significantly less subcutaneous, omental, and hepatic fat in GIP mAb–treated animals, and the lipid profile was distinctly improved in treated mice. Glucose tolerance was markedly abnormal in control mice, whereas despite causing a marked decrease in the postprandial insulin response, glucose tolerance remained normal in GIP mAb–treated mice. These studies support the hypothesis that a reduction in GIP signaling might provide a useful method for the treatment and prevention of obesity.

METHODS

**Animals.** Eight-week-old male C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were acclimated for one wk before the initiation of all studies and were kept at 22 ± 2°C on a 12:12-h light-dark cycle and had access to food and water ad libitum unless noted otherwise. Mice were housed in groups of five per cage until reaching a weight of 30 g, at which time they were housed two to three animals per cage. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University.

**Generation of hybridomas.** GIP mAbs were produced in conjunction with The Hybridoma Core Facility at the Lerner Research Institute (The Cleveland Clinic Foundation, Cleveland, OH). A peptide with the sequence CLLAQRGGKSDKWHKNTQ, corresponding to the last 17 amino acids of mature mouse GIP-(1–42), with a cysteine residue added to the NH₂ terminus, was synthesized and conjugated to keyhole limpet hemocyanin. The conjugate was then used to inject a group of four mice at weeks 1, 4, 7, 10, and 24, after which spleens were harvested, and splenocytes were isolated before B-cells were fused with SP2/0 myeloma cells in vitro to generate hybridomas, following standard methods (20). The hybridoma clones were then diluted and transferred to 96-well plates at a density of ~1 cell/well. Hybridoma cells were expanded for several days before supernates were collected and screened for GIP binding activity using ELISA, as described in the next section.

**Screening for GIP-specific mAbs by ELISA.** MaxiSorp plates (96-well; Thermo Scientific, Grand Island, NY) were coated with synthetic mouse GIP-(1–42) (Phoenix Pharmaceuticals, Burlingame, CA) using 50 μg/ml of a 4 μg/ml solution in 0.2 M carbonate-bicarbonate buffer, pH 9.4. After incubation overnight at 4°C, wells were washed with PBS before hybridoma supernates were added for 4 h at 37°C. The supernates were then aspirated and the wells washed with 0.4 mg/ml BSA in PBS before a solution of goat anti-mouse IgG conjugated with horseradish peroxidase (HRP, Jackson Laboratories) diluted 1:5,000 was added. After incubation for 1 h at 37°C, the solution was removed, and the wells were washed before HRP content was detected using a 4 mg/ml solution of o-phenylenediamine dihydrochloride in 0.4 mg/ml urea hydrogen peroxide, and 0.05 M phosphate-citrate, pH 5.0. The reaction was stopped by the addition of an equal volume of 4 N sulfuric acid, and HRP activity was quantified by measuring absorbance at 490 nm.

**Identification of mAbs that bind to GIP in suspension.** Supernates that scored positive for GIP binding activity by ELISA were subsequently tested with 14 μg/ml solution of mouse GIP in PBS for 30 min at 37°C before the mixture was subjected to ELISA, as above. Wells with low HRP activity corresponded to mAbs that bound effectively to GIP in suspension, and corresponding hybridoma clones were selected for further screening.

**Identification of mAbs that neutralize GIP in vitro.** The ability of hybridoma supernates to neutralize GIP and prevent ligand-receptor interaction, receptor activation, and receptor-dependent signaling was tested using the GIPR-expressing reporter cell line LGIPR2 (43). This reporter cell line contains the LacZ gene under the control of the Camp-dependent vasoactive intestinal peptide (VIP) promoter and was derived by transfecting LVIP cells with the rat GIPR, as previously described (43). LGIPR2 cells are activated by GIP in a concentration-dependent manner (43). Supernates were diluted 1:1 and 1:20 with 1 nM solutions of mGIP and added to LGIPR2 cells for 4 h before the cells were assayed for reporter activity, as previously described (43).

**Purification of IgG from hybridoma cells.** Hybridoma cells producing GIP-neutralizing mAbs were adapted to growth in serum-free medium, mixed with a 14 μg/ml solution of mouse GIP in PBS for 30 min at 37°C before the mixture was subjected to ELISA, as above. Wells with low HRP activity corresponded to mAbs that bound effectively to GIP in suspension, and corresponding hybridoma clones were selected for further screening.

In vitro response to GIP and glucagon in the presence of increasing concentrations of GIP mAb. To demonstrate that the GIP mAb specifically neutralized GIP activity, purified IgG at concentrations of 0, 1.56, 3.13, 6.25, 12.5, or 25 μg/ml were mixed with increasing amounts (0, 0.001, 0.01, 1.0, 10.0, and 100.0 nM) of mGIP or glucagon (gcg; human, mouse, rat, porcine, bovine, canine; Phoenix Pharmaceuticals, Burlingame, CA) in DMEM containing 10% FBS. After incubation at 37°C for 15 min, the mixtures were added to LGIPR/gcgR reporter cells. These reporter cells, which respond to both GIP and gcg by increasing LacZ gene expression, were derived by the transduction of LVIP cells (42, 43) with lentiviral pseudoparticles containing cDNAs encoding the mouse GIPR (mGIPR) and the mouse glucagon receptor (mgcgR). The cells were incubated for 4 h at 37°C before the mixtures were removed, and the cells were then washed and reporter activity was assayed.

**Affinity determination by surface plasmon resonance.** Binding experiments were performed using a Biacore 3000 instrument with CM5 sensor chips (GE Healthcare Life Sciences, Pittsburgh, PA). The purified mAb (ligand) was immobilized using an amine couple kit (GE Healthcare). GIP (mouse, human, and rat) and glucagon-like peptide-1 (GLP-1; the analytes) solutions from 0.05 to 1.000 nM were prepared in 0.01 M HEPES, pH 7.4, 0.15 M NaCl with 0.005%
Inhibition of the insulin response to oral glucose using a GIP-specific mAb. Two groups of 5- to 9-wk-old C57BL mice were fasted for 5 h before either GIP mAbs (30 mg/kg BW) or vehicle (PBS) was administered intravenously. Sixty minutes later (time 0), 50 μl of blood was collected from the saphenous vein of each mouse before 2 g/kg BW glucose was administered by oral gavage. Additional 50-μl aliquots of blood were collected 15 and 30 min later from the periorbital sinus after the mice were anesthetized using isoflurane. Alternate eyes were used for each collection. Blood glucose levels were measured using a TrueTest glucometer (NIPRO Diagnostics, Fort Lauderdale, FL), and serum insulin levels were measured using a specific ELISA (ALPCO, Salem, NH).

Inhibition of GIP-dependent insulin response in vivo. Three groups (n = 4) of mice were administered either 30 mg/kg BW mAbs (treated) or vehicle (2 untreated groups) by ip injection and were then fasted for 6 h. After the fast (time 0 min), 50 μl of blood was collected from the saphenous vein of each animal. The treated group and one untreated group were administered 2 g/kg BW glucose + 2.5 nmol hGIP/kg BW by ip injection. The other untreated group of mice was administered 2 g/kg BW glucose only. Fifty microliters of blood was collected at 15 and 30 min, and serum insulin content was measured, as above.

Diet study. Thirty mice were divided randomly into three groups of 10 mice each. Two groups were fed a HFD and one group a low-fat (LF) diet (LFD). Both chows were purchased from Harlan-Teklad (Indianapolis, IN). The HFD (catalog no. TD.06414) consisted, by weight, of ~23.5% protein, 27.3% carbohydrates, and 34.3% fat. The HFD provided ~18.4% of total calories from protein, 21.3% from carbohydrates, and 60.3% from fat, and 5.1 kcal/g. The LFD (catalog no. TD.08806) consisted, by weight, of ~18.6% protein, 62.6% carbohydrates and 4.2% fat, and it provided 20.5% of total calories from protein, 69.1% from carbohydrates, and 10.4% from fat. All mice had access to food and water ad libitum throughout the study.

One group of mice (HFD-control) was fed the HFD for a total of 17 wk and administered 0.1 ml of vehicle (PBS) by ip injection 5 times/wk. The second group (HFD-mAb) was fed the HFD for a total of 17 wk and administered GIP mAbs 5 times/wk; the total dose of the mAb was 60 mg·kg BW·wk⁻¹. The third group was fed the LFD (LFD-control) for the 17 wk and received no injections. The weight of each mouse was measured and recorded each Monday evening, and the amount of food consumed was also measured and recorded throughout the course of the study.

Imaging studies. After week 17 of the diet study, six HFD-control mice and six HFD-mAb mice were subjected to magnetic resonance imaging (MRI). Specifically, a 2-D, T1-weighted asymmetric echo, Rapid Acquisition with Relaxation Enhancement (aRARE) sequence was performed using a Bruker Biospec 7T small animal MRI scanner (Bruker Biospin, Billerica, MA) (TR = 1.087 ms, TE = 9.1 ms, T2 = 23.5 ms).

Fig. 1. Characterization of a gastric inhibitory polypeptide (GIP)-specific monoclonal antibody (mAb). Left: effect of increasing concentrations of GIP-mAb on mGIP (A) and glucagon (gec; B) dose-response experiments using reporter cells in vitro. Right: binding of analytes [mGIP and glucagon-like peptide-1 (GLP-1)] to ligand (GIP mAb) was detected by surface plasmon resonance (SPR). Representative association and dissociation sensograms from binding analysis of mGIP (C) and GLP-1 (D) to immobilized GIP-mAb.

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FOV = 100 × 50 mm, 512 × 256 matrix, 4 averages, echo train length = 4, slice thickness = 1 mm). The images obtained from the aRARE acquisition were used to generate separate fat and water images by a modified IDEAL image reconstruction technique for 7T and allowed for determination of lipid biodistribution in the mice, as previously described (17).

**Qualitative analysis of liver fat content.** Upon completion of the diet study, mice were euthanized, and the livers were isolated, embedded in OCT and flash-frozen in 2-methyl-butane dry ice bath, and then stored at −80°C. For microscopic analysis, 10-μm cryosections (Leica CM1850) were prepared on Superfrost Plus slides (Thermo Fisher Scientific). After a 1-h fixation in ice-cold 4% paraformaldehyde, slides were washed once in PBS and three times in deionized water (dH2O) before staining with two parts 0.5% Oil Red O stain (Sigma-Aldrich, St. Louis, MO) to three parts dH2O at 60°C for 10 min. Slides were then washed with dH2O, counterstained with Hematoxylin QS (Vector Laboratories, Burlingame, CA) for 30 s, and washed again with dH2O before a coverslip was affixed with PBS-

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**Fig. 2.** Effect of a GIP-specific mAb on the insulin response and glucose tolerance in mice. A–D: mice (n = 5/group) were fasted for 4–5 h before GIP mAbs (30 mg/kg BW) or vehicle were injected iv 1 h prior to administration of oral glucose. Immediately prior to and after (10 and 30 min) glucose gavage, 50 μl of blood were collected from each mouse, and serum glucose and serum insulin were measured. A: time course of insulin response to oral glucose in mice with and without GIP mAb. B: incremental area under the curve (iAUC) for the insulin response (*P < 0.01). C: oral glucose tolerance tests (OGTTs) in mice with and without GIP mAb. D: iAUC for the OGTTs. E and F: 3 groups (n = 4) of mice were administered 30 mg/kg BW mAbs ip (glucose + hGIP + GIP mAb) or vehicle (glucose ± hGIP) and then fasted for 6 h. After the fast (time = 0 min), glucose with and without hGIP was administered ip, and 15 and 30 min later, 50 μl of blood was collected from each animal and serum insulin content measured. E: time course of insulin response in mice to ip glucose in the presence and absence of hGIP with and without GIP mAb. Results are expressed as mean insulin concentration (ng/ml) ± SE. F: iAUC for the insulin response. Values are expressed as means ± SE in ng·min·ml⁻¹. *P < 0.05, glucose + hGIP vs. glucose + hGIP + mAb.
glycerol. Images were captured immediately with a Nikon E600 microscope with Spot RT camera and software (Diagnostic Instruments, Sterling Height, MI).

**Glucose tolerance tests.** At the end of the diet study (week 17), six mice from each group were fasted for 6 h before 2 g/kg BW glucose was administered ip. Immediately prior to, and at 15, 30, 60, and 120 min after glucose injection, ~2 μl of blood was collected from the tail vein of each mouse, and blood glucose concentration levels were measured, as above.

**Biochemical measurements.** After animals were euthanized at the end of the diet study, whole blood was collected, serum was separated, and biochemical analyses were performed. Serum insulin, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), leptin, adiponectin, gcg, and total GLP-1 concentrations were measured by the Mouse Biology Program (University of California, Davis, Sacramento, CA). Serum TG, TC, LDL, and HDL levels were measured using enzymatic assays (Wako Diagnostics, Richmond VA), while serum insulin, leptin, adiponectin, and GLP-1 concentrations were measured using electrochemiluminescence assays (Meso Scale Discovery, Rockville, MD). Serum gcg levels were measured by radioimmunoassay (EMD Millipore, Billerica, MA).

**Statistical analysis.** All results are expressed as means ± SE. Statistical analysis was performed using two-way analysis of variance (ANOVA; BMDP Statistical Software, Los Angeles, CA) and Student’s t-test for unpaired values. P ≤ 0.05 was considered to be statistically significant.

**RESULTS**

**Generation and in vitro characterization of a GIP-neutralizing mAb.** During the initial screening of supernates from ~2,000 hybridoma clones, 21 scored positive for GIP binding, as determined using a specific ELISA. However, only five clones produced mAbs with sufficiently high binding affinity to mouse GIP (mGIP) in suspension. Supernates from these hybridomas were diluted 1:1 and 1:20 with solutions of mGIP and were then added to cAMP-dependent reporter cells expressing the rat GIPR. All five supernates inhibited GIP-dependent reporter activity by at least 40% when diluted 1:1, and the supernate from one hybridoma clone (GIP-mAb) showed significant inhibition when diluted 1:20 (Data not shown). This clone was expanded, and IgG was isolated using thiolphilic adsorption chromatography. Purified IgG neutralized 1 nM mGIP, but not gcg, in a concentration-dependent manner and prevented induction of a cAMP-dependent reporter gene in cells expressing mGIPR and mcrgR. As depicted in Fig. 1, A and B, the addition of increasing amounts of GIP mAb caused a rightward shift in mGIP, but not gcg, dose-response curves. The purified mAb was also able to neutralize human GIP-dependent signaling in the same reporter system (data not shown). SPR was used to analyze binding interactions between the mAb (ligand) and GIP from mouse, human (h)GIP, and rat (r)GIP and to determine the equilibrium dissociation constant (K_D) for each binding pair. The calculated K_D’s were 4.1, 3.3, and 4.9 nM for mGIP, hGIP, and rGIP, respectively. GLP-1 binding to the GIP mAb was also evaluated using SPR, and no specific binding was detected. Representative sensograms resulting from SPR analysis of the interactions between the ligand GIP-mAb and the analytes mGIP and GLP-1 are presented in Fig. 1, C and D, respectively.

**Effect of a GIP-neutralizing mAb on oral glucose tolerance.** Inhibition of GIP-dependent insulin secretion was used as an endpoint to evaluate the GIP mAb in mice. In response to oral glucose gavage, serum insulin levels of control animals increased significantly (Fig. 2, A and B). The iv administration of the GIP-mAb 1 h prior to glucose gavage markedly diminished the insulin response (Fig. 2A). The incremental area under the curve (iAUC) for the group pretreated with the GIP mAb was 3.3-fold less than for the control group (P < 0.01; Fig. 2B). No significant differences were detected in

![Fig. 3. Effect of GIP-specific mAb on weight gain in mice fed a high-fat diet (HFD). Mice (n = 10/group) were fed a HFD and treated with vehicle (HFD-control) or a GIP-specific mAb (HFD-mAb) or were fed an LFD (LFD-control). A: mice were weighed every week for 17 wk. *P < 0.001 vs. HFD-mAb. Inset: absolute weight gain over the 17-wk diet for mice fed the HFD and treated with vehicle (HFD-Control) or with the GIP-specific mAb (HFD-mAb). Results are expressed as means ± SE. B: amount of chow consumed by mice in each group between week 1 and week 17. NS, not significant. **P < 0.05 vs. HFD-mAb. Results are expressed as means ± SE.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00345.2015)
blood glucose concentrations between the control mice and GIP mAb-treated mice (Fig. 2, C and D). Similar results were observed when the GI mAb was administered ip 5 h prior to glucose gavage (data not shown).

**Effect of a GIP-neutralizing mab on the insulin response to ip coadministration of glucose and hGIP.** To demonstrate that the GIP mAb could neutralize hGIP in vivo, GIP mAbs or vehicle was administered ip 6 h prior to the ip coadministration.

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**Fig. 4.** Treatment of mice fed a HFD reduces fat deposition in mice. **A:** representative MRI from HFD-control (left) and HFD-mAb mice (right) at week 17. **B:** subcutaneous and omental fat content measured using the relaxation compensated fat fraction (RCFF) \( (n = 6/\text{group}) \); \* \( P < 0.001 \). **C:** total body fat (omenta + subcutaneous) and lean body weight (BW = omental + subcutaneous) of HFD-control and HFD-mAb mice. **D:** photograph of abdominal cavity of representative HFD-control (left) and HFD-mAb mice (right). Green/blue color apparent in the intestine is due to the dye included in HF chow. **E:** muscle and hepatic fat content in HFD-control and HFD-mAb mice measured using RCCF \( (n = 6/\text{group}; \* P = 0.09, ** P = 0.03) \). **F:** representative photomicrograph of liver sections stained with Oil Red O in HFD-control (left) and HFD-mAb mice (right). Results are expressed as means ± SE.
of glucose and hGIP. As an additional control, untreated mice administered ip glucose without hGIP were also evaluated. Glucose injection alone resulted in only a small increase in the insulin response, whereas the addition of hGIP increased the response significantly (Fig. 2E). Pretreatment of mice with GIP mAbs increased the insulin response similarly to the mice administered glucose without hGIP (Fig. 2E). The iAUC for the group pretreated with mAb and administered glucose plus hGIP was 3.4-fold lower than the iAUC for the group pretreated with vehicle and coadministered glucose and hGIP ($P < 0.05$; Fig. 2F).

**Effect of GIP mAb on weight gain.** After 1 wk of the diet study, both HFD-control and HFD-mAb mice had gained ~10% of their body weight. The weight gains of both HFD groups remained nearly identical until week 4, when weight gain appeared to plateau in the HFD-mAb group, whereas the weights of the HFD-control group continued to steadily increase (Fig. 3). The difference in weight gain between the two groups became significant ($P < 0.001$) by week 7. After 17 wk on the diet, HFD-control mice had gained an average of $21.5 \pm 1.0$ g, while HFD-mAb mice had gained an average of $11.5 \pm 0.5$ g (Fig. 3, inset), a reduction in weight gain of 46.5% ($P < 0.001$). The amount of chow consumed per mouse through week 17 for HFD-control and HFD-mAb mice was $330 \pm 22$ and $296 \pm 2$ g (means ± SE), respectively ($P > 0.2$). In addition, when corrected for body weight at week 16, the HFD-control mice had consumed less chow than the HFD-mAb mice ($7.4 \pm 0.7$ and $8.9 \pm 0.5$ g/BW, respectively), a difference that was not significant ($P > 0.2$). The HFD-control mice gained weight at a slower rate throughout the duration of the study. The HFD-mAb group and the LFD-control group gained approximately the same amount of weight after 17 wk on their respective diets (Fig. 3). Although not quantified, mice in all three groups appeared healthy throughout, and no obvious differences in physical activity were observed.

**Effect of GIP mAb on fat deposition.** A representative MRI of a mouse from the HFD-control and HFD-mAb groups at week 17 is shown in Fig. 4A. The fat content in all areas was significantly higher in the HFD-control group than in the HFD-mAb group. Quantification using the relaxation compensated fat fraction (RCFF), showed that HFD-control animals possessed ~2-fold more subcutaneous fat ($20.06 \pm 1.30$ vs. $10.17 \pm 0.77$ g, $P < 0.001$) and ~3.5-fold more omental fat ($9.28 \pm 0.52$ vs. $2.68 \pm 0.53$ g, $P < 0.001$) than HFD-mAb mice, respectively (Fig. 4B). Moreover, the lean body weights of HFD-control and HFD-mAb mice were $16.45 \pm 1.00$ and $22.79 \pm 0.71$ g, respectively ($P < 0.001$; Fig. 4C). Visual inspection of the abdominal cavities after partial dissection clearly showed significant accumulation of fat in HFD-control animals, with omental fat in the HFD-mAb animal being nearly absent (Fig. 4D). The muscle fat contents in HFD-control and HFD-mAb mice were $0.088 \pm 0.012$ and $0.061 \pm 0.007$ g, respectively ($P = NS$, Fig. 4E), representing a 1.4-fold difference ($P = NS$). The hepatic fat contents in HFD-control and HFD-mAb mice were $0.311 \pm 0.05$ and $0.145 \pm 0.03$ ml, respectively (Fig. 4E), representing a 2.1-fold difference ($P < 0.05$). In addition, as clearly depicted in Fig. 4F, a marked decrease in hepatic fat accumulation, in terms of both the number and the size of fat droplets, was evident in the livers of HFD-mAb mice compared with HFD-control mice after staining with the lipophilic dye Oil Red O.

**Glucose tolerance tests and biochemical measurements.** During the performance of ip glucose tolerance tests at the end of the 17-wk study period, serum glucose levels in the HFD-control mice increased far greater than in HFD-mAb and LFD-control mice (Fig. 5). In addition, the serum glucose levels in HFD-mAb mice and LFD-control mice decreased more rapidly than in HFD-control mice, consistent with the development of insulin resistance in HFD-control mice, but not in HFD-mAb or LFD-control mice.

Hyperinsulinemia developed in HFD-control mice, with a fasting insulin level of $4.29 \pm 1.10$ ng/ml; the insulin level in HFD-mAb mice was $1.65 \pm 0.55$ ng/ml, 2.6-fold less than HFD-control mice ($P = 0.05$) and similar to that measured in the LFD-control group (Fig. 6A). Similarly, serum leptin levels detected in HFD-mAb mice were reduced by >80% compared with levels detected in HFD-control animals ($6.5 \pm 1.9$ vs. $37.5 \pm 10.4$ ng/ml, $P = 0.01$; Fig. 6B); serum leptin levels in LFD-control mice were similar to those measured in HFD-mAb mice. No significant differences were detected in either serum adiponectin, glg, or GLP-1 levels among any of the three groups of mice (Figs. 6, C–E).

The lipid profile in the HFD-mAb mice compared with the HFD-control group was markedly improved. After the 17-wk study period, serum TG levels were lower in treated mice than in controls ($56.2 \pm 6.8$ vs. $81.1 \pm 6.4$ mg/dl, $P < 0.05$; Fig. 7A). The serum TC and LDL levels were also lower in the HFD-mAb group compared with the HFD-control group. Serum TC levels were $147.0 \pm 15.7$ and $227.8 \pm 25.9$ mg/dl ($P < 0.05$, Fig. 7B), whereas serum LDL levels were $48.7 \pm 3.7$ and $95.1 \pm 15.4$ mg/dl ($P < 0.01$; Fig. 7C) in HFD-mAb and HFD-control mice, respectively. Although HDL levels were lower in HFD-mAb mice, the HDL/TC ratio in this group was significantly greater in the HFD-control group ($0.266 \pm 0.005$ vs. $0.213 \pm 0.021$, $P < 0.05$; Fig. 7D). With the
exception of the HDL/TC ratios, the lipid profile in the HFD-mAb group was similar to that detected in the LFD-control group (Fig. 7, A–D).

**DISCUSSION**

In the current study, we have identified and characterized a mouse mAb that binds GIP and attenuates signaling in vitro and in vivo. The binding data obtained using SPR indicated that the mAb interacts with mGIP, hGIP, and rGIP with similar affinities, suggesting that the mAb will effectively neutralize GIP signaling in a number of different species. The mAb inhibited insulin secretion stimulated by the concomitant administration of hGIP and glucose and by endogenous mGIP. Despite the significant reduction of insulin following oral glucose, there was no significant difference in glucose excursion in mice over the first 30 min. Similar results were previously reported in some (19, 27, 41), but not all (23), studies in which GIP signaling was reduced before the perfor-

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GIP promotes fat deposition, as well as the mechanisms accounting for its prevention by reducing GIP signaling, has not yet been elucidated. However, the regulation of glucose absorption and the insulin response by GIP appear to be of paramount importance. The current study is consistent with diet studies in rats in which investigators demonstrated the prevention and reversal of excessive weight gain when a regular HFD was substituted with a high-fat/low-carbohydrate diet (18).

The prevention of obesity in mice with reduced GIP signaling has been associated with higher fat oxidation (22, 27). However, it is unclear whether GIP directly modulates fatty oxidation and energy expenditure or whether the observed changes arise indirectly as a result of other biological properties of GIP. As mentioned above, GIP increases the postprandial insulin response and intestinal glucose absorption. Both of these effects would be expected to lead to increased glucose uptake and utilization in liver, fat, and peripheral tissues, thereby enhancing lipogenesis (11). In contrast, the lower levels of postprandial glucose and insulin occurring as a result of the inhibition of GIP signaling might lead to a greater utilization of fat as an energy source, as well as an associated increase in fatty oxidation (25).

Nonalcoholic fatty liver disease has become a major health issue, and in the current study, the attenuation of GIP signaling markedly attenuated fat accumulation in the liver (Fig. 4, E and F). In HFD-control animals, higher circulating levels of fatty acids and glucose are thought to promote fatty acid and TG uptake in the liver and inhibit β-oxidation, resulting in hepatic fat accumulation. In addition, elevated insulin and glucose increase the hepatic expression of genes involved in de novo lipogenesis, primarily by activating the transcription factors sterol regulatory element-binding protein-1c and carbohydrate-responsive element-binding protein, respectively (3). Therefore, decreased hepatic fat in mice with reduced GIP signaling may have resulted from lower levels of postprandial plasma glucose and insulin and the associated decrease in the activity of the biological processes that stimulate de novo hepatic lipogenesis.

The methods that have been evaluated to date to attenuate GIP signaling, neutralizing mAbs, vaccination against GIP (7, 15, 16, 24), and peptide antagonists (8) all possess benefits and limitations. Neutralizing mAbs and peptide antagonists (8) are highly specific and can be immediately effective once delivered to the animal. However, they are both expensive and require repeated administration to be effective. Vaccination (7, 24) would be less expensive and would require only the few vaccine injections needed to increase the antibody titer to a level sufficient to neutralize GIP. Vaccination would not, however, be effective immediately, since it would likely require weeks to months for titers to reach levels necessary to reduce GIP signaling. Moreover, because each animal must elicit its own immune response to the vaccine, the potential for greater variability would likely be significant. In addition, a vaccine would likely lead to the irreversible reduction in GIP signaling. While some reports have demonstrated the value of Pro3-GIP as a peptide antagonist both in vitro and in vivo (9, 21), two studies by other investigators failed to demonstrate benefit in vivo (32, 38). We have recently examined Pro3-GIP (Phoenix Pharmaceuticals, Burlingame, CA) and found it to be a weak agonist that could antagonize GIPR in vitro but only at concentrations more than 100-fold higher than previously reported (unpublished data). The reasons for the disparate results are unclear but may be related to differences in methods for preparing the peptide or differences inherent in the in vitro assays used by the different investigators.

**Fig. 7. Effect of GIP mAb on lipid profile.** After the 17 wk diet period, HFD-control (n = 4/group), HFD-mAb (n = 5/group), and LFD-control (n = 5/group) were fasted for 6 h; then blood was collected and serum lipids were measured. A: serum triglycerides (TG; mg/dl): *P < 0.05 vs. HFD-control group. B: serum total cholesterol (TC; mg/dl): *P < 0.05, **P = 0.071, both vs. HFD-control group. C: serum LDL (mg/dl): *P < 0.05, both vs. HFD-control group. D: serum HDL/TC ratio: *P < 0.05 vs. HFD-control group. Results are expressed as means ± SE.
In contrast to the use of peptides, the specificity inherent to mAbs, along with the emergence of more efficient methods for the generation of antibodies and antibody-derived molecules, mAbs, along with the emergence of more efficient methods for the generation of antibodies and antibody-derived molecules, mAbs, will likely expand their use for treating human disease, including obesity. The dose of antibody administered in the current study, 60 mg·kg BW \(^{-1}\)·wk \(^{-1}\), likely represents an excess amount. To prove the hypothesis regarding the attenuation of GIP signaling and its effects on body weight (22), we administered the GIP mAb in an amount sufficient to inhibit insulin release (Fig. 2). Future studies will require formal pharmacokinetic and pharmacodynamic analyses to determine the optimal amount necessary for clinical utility. In addition, although nonspecific mouse IgG would have been preferable as a control in place of PBS, the cost of mouse IgG was prohibitive.

Our initial intent was to perform a study of 16–20 wk duration. The difference in weight gain between the two groups became significant (P < 0.001) by week 7. After 17 wk on the diet, HFD-mAb mice had gained 46.5% less weight than HFD-control mice (Fig. 3; inset; P < 0.001), and we accordingly elected to terminate the study at that point. Although we are unable to determine direct effects of the GIP mAb, it is likely that the decreased weight gain associated with its use accounted for the reduction in whole body, omental, hepatic, and subcutaneous fat rather than the addition of excess IgG itself. Finally, although no obvious differences in physical activity were observed, it will necessary to perform formal studies in the future to specifically assess the effects of the GIP mAb on oxygen consumption in the resting and active states.

In contrast to the evidence for the beneficial effects of disruption in GIP signaling, Finan et al. (6) synthesized a dual incretin peptide agonist and demonstrated beneficial metabolic benefits in rodents, monkeys, and humans. They showed that this peptide enhanced the antihyperglycemic and insulinotropic benefit conferred by selective GLP-1 agonists. This dual peptide also reduced fat mass in obese rodents, whereas a GIP-selective agonist had no such effect. It also corrected obesity-related insulin resistance and pancreatic insulin deficiency, two of the etiologic mechanisms responsible for “diabetes” (6, 38).

The results of the current studies have demonstrated the marked benefit of a GIP mAb in attenuating diet-induced weight gain in mice. These effects were independent of food consumption, and the principal benefit appeared to be the striking decrease in fat deposition in the liver, omentum, and subcutaneous space. These studies support the hypothesis that a reduction in GIP signaling by the use of a specific GIP mAb might provide a useful method for the treatment and prevention of obesity and related disorders.

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